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Grief Symptoms Promote Inflammation During Acute Stress Among Bereaved Spouses

Ryan L. Brown[1], Angie S. LeRoy[1], Michelle A. Chen[1], Robert Suchting[2], Lisa M. Jaremka[3], Jia Liu[4], Cobi Heijnen[4], and Christopher P. Fagundes[1,4,5]

[1]Department of Psychological Sciences, Rice University; [2]Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston; [3]Department of Psychological and Brain Sciences, University of Delaware; [4]Department of Behavioral Sciences, The University of Texas MD Anderson Cancer Center; and [5]Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine

Abstract

The death of a spouse is associated with maladaptive immune alterations; grief severity may exacerbate this link. We investigated whether high grief symptoms were associated with an amplified inflammatory response to subsequent stress among 111 recently bereaved older adults. Participants completed a standardized psychological stressor and underwent a blood draw before, 45 min after, and 2 hr after the stressor. Those experiencing high grief symptoms (i.e., scoring > 25 on the Inventory of Complicated Grief) experienced a 45% increase in interleukin-6 (IL-6; a proinflammatory cytokine) per hour, whereas those experiencing low grief symptoms demonstrated a 26% increase. In other words, high grief was related to a 19% increase in IL-6 per hour relative to low grief. The grief levels of recently bereaved people were associated with the rate of change in IL-6 following a subsequent stressor, above and beyond depressive symptoms. This is the first study to demonstrate that high grief symptoms promote inflammation following acute stress.

Keywords

aging, death and dying, stress reactions, health, depression, grief, bereavement

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Spouses represent the closest and most significant enduring relationship for most adults (Doherty & Feeney, 2004). The spousal bond is characterized by functional interdependence and a state of physiological coregulation (Proulx et al., 2007; Robles et al., 2014; Uchino et al., 1996). The coregulatory processes once maintained by the attachment relationship are disrupted when a spouse dies (Sbarra & Hazan, 2008). Numerous conceptual models and a growing number of empirical studies underscore the premise that grief temporarily dysregulates multiple bodily systems, promoting a state of "biological dysregulation"—a term we adopt from the seminal work by Hofer (1984) and Sbarra and Hazan (2008). This state of biological dysregulation alters the stress-response system, which should theoretically amplify a bereaved spouse's physiological stress response (Kiecolt-Glaser et al., 2020). Maladaptive immune alterations are one marker of biological dysregulation.

Bereavement is reliably associated with maladaptive immune alterations, such as elevated basal proinflammatory immune signaling. Proinflammatory cytokines, specifically, are signaling molecules released from immune cells and are responsible for both local and systemic inflammation. Stress and depression boost levels of proinflammatory cytokines (e.g., to acute stress;

Corresponding Author:
Christopher P. Fagundes, Rice University, Department of Psychological Sciences
Email: christopher.fagundes@rice.edu

Fagundes et al., 2013). In the bereavement literature, proinflammatory cytokines and inflammatory indices have received considerable attention because (a) an overactive inflammatory network contributes to many diseases of older adulthood (Rea et al., 2018) and (b) bereavement is more common in older adulthood. Specifically, interleukin-6 (IL-6) is a primary target for clinical interventions (Hunter & Jones, 2015). Across three separate studies, basal levels of circulating IL-6 cytokines were higher among bereaved spouses than matched comparison subjects; IL-6 was reliably higher in all three studies (Cankaya et al., 2009; Cohen et al., 2015; Schultze-Florey et al., 2012). These data match related findings showing that IL-6 is the cytokine that most reliably increases when people are exposed to stress (Marsland et al., 2017; Steptoe et al., 2007).

Chronic negative emotions, such as stress and depression, promote biological dysregulation, which in turn sensitizes (i.e., primes) the inflammatory stress response (Fagundes et al., 2013; Kiecolt-Glaser et al., 2020; Sbarra & Hazan, 2008). Experimental work in humans has shown that chronic negative emotions prime the inflammatory stress response, boosting reactivity to experimentally manipulated laboratory stressors. For example, among healthy adults, those with more depressive symptoms produced a greater IL-6 response to an experimental stressor than those who reported fewer depressive symptoms (Fagundes et al., 2013). Similarly, among both healthy adults and post-treatment breast cancer survivors, those who were lonelier experienced a greater synthesis of IL-6 by peripheral blood mononuclear cells when stimulated with lipopolysaccharide (a bacterial endotoxin) in response to an acute stressor (Jaremka et al., 2013).

There are multiple studies showing that, compared with those who are not bereaved, people who have lost a spouse exhibit elevated markers of proinflammatory cytokines (Cohen et al., 2015) and produce more proinflammatory cytokines when leucocytes are stimulated by lipopolysaccharide (Fagundes et al., 2018). Among bereaved spouses, those experiencing more severe grief symptoms also produced more proinflammatory cytokines by peripheral blood leukocytes when stimulated with lipopolysaccharide (Fagundes et al., 2018). A subgroup of people (~10%) experience intense and persistent grief (Prigerson et al., 2009), which may sensitize the immune system to respond more aggressively to future acute stress (similar effects have been found in previous studies of depression; Fagundes et al., 2013; Pace et al., 2006),

During bereavement, symptoms characteristic of either grief or depression (or both) are common (Bonanno et al., 2002; Zisook & Shear, 2009). Depression and grief have distinct and overlapping symptoms, which are both characterized by a state of biological dysregulation. For example, negative affect is present in both experiences; however, grieving bereaved spouses do not generally report low self-esteem and worthlessness, which are essential aspects of depression (Friedman, 2012). Additionally, unlike depression, grief is characterized by intense separation distress (LeRoy et al., 2019; Shear et al., 2005, 2011).

In the current study, we investigated whether grief amplifies recently bereaved spouses' acute inflammatory response to an experimental laboratory stressor, independently of depressive symptoms. We hypothesized that bereaved spouses who experienced high grief symptoms (operationalized using a standard cut score) would exhibit a more exaggerated increase in IL-6 following an acute stressor than bereaved spouses who experienced low grief symptoms. On the basis of established literature showing that depressive symptoms sensitize the inflammatory stress response to acute stressors, we were interested in whether grief would sensitize the inflammatory stress response after accounting for depressive symptoms.

Statement of Relevance

It is now well established that when one spouse dies, the surviving spouse is at increased risk of dying in the following year; this is colloquially known as the "widowhood effect." Because negative emotions profoundly affect people's physiology, the stress and intense grief that sometimes accompany the loss of a spouse may contribute to the widowhood-effect phenomenon. One hundred eleven recently widowed older adults engaged in a 15-min standardized laboratory task that elicits psychological stress. Blood was taken before the stressor and twice over the next 2 hr to evaluate a critical immune marker associated with aging and disease (i.e., the proinflammatory cytokine interleukin-6 [IL-6]). We sought to determine whether grief-symptom severity impacted the IL-6 response that typically occurs in people after they experience a brief stressor. We found that levels of IL-6 increased 19% more among bereaved spouses who reported higher levels of grief than among those who reported lower levels of grief.

Method

Participants

A power analysis indicated that a minimum of 89 total participants was necessary to achieve 80% power to

detect a small- to medium-sized effect (selected on the basis of a previous study using the same experimental method; Pace et al., 2006) when employing the traditional .05 criterion of statistical significance and accounting for all covariates included in the adjusted model. Approximately 4 months after the death of their spouse (M = 138 days; SD = 17 days), 111 participants completed this study, which was conducted between February 2016 and August 2018. The current study was part of a larger longitudinal observational study investigating the biological mechanisms underlying greater risk of cardiovascular disease risk during bereavement.

For the parent study, we contacted and recruited people who recently experienced the death of their spouse from obituaries, support groups, flyer distribution, online postings, and community events. All recruitment strategies were approved by the local institutional review board. Exclusion criteria included having significant visual or auditory impairment, being pregnant or nursing, having autoimmune and inflammatory diseases, having experienced bereavement because of loss of another loved one in the last year, and getting divorced within the past year. We recruited only English speakers to ensure understanding of the questionnaires. On the basis of research establishing that it takes 3 years for individuals to show a partner preference for attachment-related functions (i.e., proximity seeking, safe haven, secure base; Fagundes & Schindler, 2012), we excluded people who had not been married for at least 3 years before the death of their spouse.

Procedure

The day before the visit, a research assistant called participants and reminded them of the next day's visit. Because inflammatory markers may be elevated during acute illnesses (e.g., upper respiratory infections), we rescheduled participants if they reported any illness symptoms (e.g., fever, congestion, sore throat, or acute infections due to injury). We also asked participants to avoid any strenuous physical activity 48 hr before all visits.

Research assistants administered anthropometric measurements, including weight, height, and waist circumference, during the early hours of the morning of the visit. After a relaxation period, a nurse inserted a catheter to draw a baseline nonfasting blood sample. Baseline samples of IL-6 were collected between 8:45 a.m. and 11:30 a.m. to control for diurnal variation. Variability in this time was due to the check-in time for each participant.

Following the baseline blood draw, participants completed the Trier Social Stress Test (TSST), an extremely well-validated laboratory social stressor that reliably enhances proinflammatory cytokine production (Fagundes et al., 2013; Jaremka et al., 2013; Kirschbaum et al., 1993). In this well-established stressor, participants were told to prepare a speech for a job interview and that they would deliver this speech to two committee members trained in behavioral observation. Participants prepared a 5-min speech about why they would be best for the job. They then gave this speech to a committee composed of research assistants who did not give the participant any positive feedback. If a participant stopped talking during the speech, the committee remained silent for 20 s. If they did not begin speaking, the chair prompted the participant to continue speaking by instructing them, "You still have time." Following this speech, participants completed a 5-min mental-arithmetic task in which they counted backward to 0 in 17-number steps, starting at 2023. When a participant made an error, the committee members corrected their mistake and asked them to start back at the beginning. Participants completed these tasks under the guise that they were being audio and video recorded and observed by the committee. The committee remained neutral and gave no positive feedback in order to maximize the social-evaluative threat present during the experience. After the TSST, participants relaxed by watching a benign Ken Burns documentary (e.g., about national parks) for the next 2 hr to standardize their experience. Blood draws also occurred at 45 min and 2 hr following the TSST. After the final blood draws, we debriefed participants before they completed questionnaires.

Measures

Grief symptoms. The Inventory of Complicated Grief (ICG) was used to assess the degree to which participants experienced grief symptoms. They answered 19 items on a frequency scale ranging from 0 (*never*) to 4 (*always*; Prigerson et al., 1995). The ICG measures 19 different grief-related symptoms (e.g., preoccupation with thoughts of the deceased, disbelief at or not accepting the death); example items include, "I feel dazed or stunned over what happened" and "I hear the voice of the person who died speak to me." Internal consistency of the 19-item ICG was high in the current sample (α = .92). People with ICG scores of 25 or more, an established clinically relevant cut score, are considered to have a high degree of grief severity (Prigerson et al., 1995), which has previously been found to be associated with worse general, mental, and physical health, social functioning, and bodily pain, as well as depression (Prigerson et al., 1995). We utilized the cut point as a case-categorical variable and our primary grief-level index on the basis of previous work showing that this

cutoff differentiated basal levels of proinflammatory cytokine production (Fagundes et al., 2019). For the remainder of the article, we refer to participants who scored above this grief cutoff as the *high-grief group* and participants who scored below this cutoff as the *low-grief group*. We also examined grief symptoms as continuous (i.e., ICG total score) as a secondary index.

Interleukin-6. Whole blood was drawn into a serum separator tube. Serum tubes were then centrifuged for 10 min at 3,000 × *g* at 4 °C. Serum aliquots were stored at −80 °C until assayed in duplicate levels for IL-6 cytokine using high-sensitivity enzyme-linked immunosorbent assays (Quantikine, R&D Systems, Minneapolis, MN) with sensitivity of detection at 0.16 pg/ml.

Depressive symptoms. The Center for Epidemiologic Studies Depression Scale (CES-D) assessed the prevalence of depressive symptoms (Radloff, 1977). The CES-D is a widely utilized measure of depression; higher scores on this scale indicate greater depressive symptomatology. The cut score for a clinically significant level of depressive symptoms is 16 (Radloff, 1977). Depressive symptoms were included in regression models as a control variable to investigate the potential unique influence of depressive symptoms on inflammation above and beyond grief symptoms in this bereaved sample because of the close association between depression and inflammation. Grief and depression are distinct constructs (Milic et al., 2017). Importantly, none of the items in the CES-D are identical to those in the ICG. There is only one conceptually similar question—"I feel lonely" (CES-D) and "I feel lonely a great deal of time ever since s/he died" (ICG)—but ICG items explicitly reference the deceased. We show what these individual models look like when depression is entered into the model as a continuous variable for our primary analyses (see Tables S1 and S2 in the Supplemental Material for results including the cut score for clinically significant depressive symptoms). The CES-D demonstrated excellent reliability in the current sample ($\alpha = .92$).

Physical activity. We assessed physical activity at this visit using the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003). The IPAQ contains items that measure the frequency and duration of vigorous-intensity activities, moderate-intensity activities, and walking during a 7-day period. We scored the IPAQ using the recommended scoring procedure (IPAQ, 2005). The respective frequency and duration values for vigorous activities, moderate activities, and walking were first multiplied together. The resulting volumes (minutes per week) of vigorous activities, moderate activities, and walking were then multiplied by their respective metabolic equivalents (METs). Finally, the resulting individual MET values were summed to form a continuous measure of physical activity in units of total MET minutes per week. We included this continuous measure of physical activity as a covariate in all adjusted analyses on the basis of recommendations to assess physical fitness using reliable surrogates (O'Connor et al., 2009), such as frequency of intense exercise, which is associated with IL-6 among older adults (Reuben et al., 2003).

Comorbid conditions. The Charlson Comorbidity Index was used to assess comorbid conditions. This is the most widely used measure to calculate a comorbidity index for predicting mortality (D'Hoore et al., 1993). The measure assigns weights to 19 physical-health-comorbid conditions on the basis of their potential influence on 1-year mortality. This was used as a covariate in all adjusted analyses, as is common in the field of psychoneuroimmunology (Fagundes et al., 2014).

Other covariates. Demographic factors (i.e., age, gender, education, days since death of spouse) and body mass index (BMI) were also included as covariates in all adjusted models on the basis of their previous associations with IL-6 and general recommendations for assessing circulating inflammatory markers (O'Connor et al., 2009). We also included the days since the death of each participant's spouse as a covariate in these analyses to control for the variability in grief that each participant may have been experiencing depending on the time since the death of their spouse. Each of these covariates was assessed at this visit time point except the time-invariant education variable, which was assessed beforehand. BMI was computed as kg/m^2. Education was assessed using an ordinal scale, starting at 0 if the participant completed graduate or professional training. This scale ranges from 0 (*graduate/professional training*) to 5 (*less than 7 years of schooling*). Total family income was assessed using an ordinal scale starting at 0 if the family did not have any income in the prior year. This scale ranged from 0 to 8 (1 = *between $5,000 and $11,999*, 8 = *$100,000 or greater*). Finally, we also included the amount of time between the baseline blood draw and the TSST as a covariate in all adjusted analyses to control for differences in time between the baseline blood draw and the TSST.

Analytic method

Preliminary statistical analysis included assessment of normality of distributions and examination for skewness and kurtosis. IL-6 values were skewed, as is normally expected for inflammatory markers (Shields et al., 2016). A natural log transformation was applied to IL-6

to better approximate a normal distribution and to satisfy the normality-of-residuals assumption, which was assessed graphically via quantile-quantile (Q-Q) plot. Because time was entered into each model in minutes, we multiplied each unstandardized coefficient by 60 to represent the increase in log IL-6 per hour to enhance interpretability. Because the outcome was log-transformed, regression coefficients for simple-slopes analyses were exponentiated to provide an index of the percentage change in the raw units of the outcome. For example, a *b* of 0.50 would reflect a change in the log outcome for a 1-unit increase in the predictor. A more readily interpretable value would be provided via exponentiation, exp(0.50) = 1.65, corresponding to a 65% increase in the raw units of the outcome (note that values below 1.0 would correspond to a decrease).

We addressed missingness on the outcome variable (i.e., inflammation) via maximum likelihood estimation. Missingness across the set of predictor variables (~2% of values) was handled via random-forest imputation in the R package *caret* (Version 6.0.86; Kuhn, 2008). In ancillary analyses, we present the findings without imputation as a sensitivity analysis for each of our hypotheses. We examined the residuals following each analysis to ensure they did not appear to deviate meaningfully from a normal distribution. Generalized linear mixed modeling, a multilevel regression analytic technique, was used to fit the outcome variable IL-6 (log-transformed) as a function of the interaction between time (minutes) and grief severity (high vs. low), controlling for constituent main effects, with a random intercept for person and a random slope for time. Person was the upper level and time was the lower level in all multilevel analyses. This model was fitted first in unadjusted fashion with no covariates and then fitted as an adjusted model with a set of fixed covariates (age, gender, years of education, body mass index, comorbidities, physical activity, days since spouse's passing, and minutes since blood was drawn before the TSST).

Prior to testing the model, we used a model-comparison approach to determine the functional form of the relationship with respect to linearity and random effects (i.e., testing whether the slope between time and inflammation varied randomly, with variation based on the individual). The −2 log likelihood was used as an index to determine whether the model with the random intercept for each individual fitted the data better than the model without the random intercept. To assess the relative quality between the models with and without random slopes, we examined (a) the Akaike information criterion (AIC; Akaike, 1973) and (b) the significance test associated with the −2 log likelihoods between the models with and without random slopes. We chose the model on the basis of a significance test between these models and after identifying the lowest AIC value, which indicates the best, most parsimonious fit with the least information lost relative to other models (Bozdogan, 1987; Vrieze, 2012). Last, we examined the relationship between time (modeled continuously in minutes) and serum IL-6 to determine whether there was a linear relationship between the two variables. We employed an unstructured within-subjects covariance matrix for all repeated measures.

All analyses were conducted in the R statistical computing environment (R Core Team, 2019). We used the package *nlme* (Version 3.1.152; Pinheiro et al., 2020) to perform multilevel analyses, *ggplot2* (Version 3.3.2; Wickham, 2016) and *ggeffects* (Version 1.0.1; Lüdecke, 2018) for visualization, and *pacman* (Version 0.5.0; Rinker & Kurkiewicz, 2017), *apaTables* (Version 2.0.8; Stanley, 2018), and *sjPlot* (Version 2.8.7; Lüdecke, 2021) to generate tables.

Results

Descriptive statistics and correlations between key variables can be found in Table 1. The mean age of the sample was 68.05 years (*SD* = 9.25), and ages ranged from 35 to 84 years old. Additionally, the sample was 65% women. Our sample consisted of 88% White participants, 6% Black participants, 3% Asian participants, and 3% participants who described their race as "other." Approximately 10% of the sample reported being Hispanic or Latino. Time since spouse's death ranged from 100 to 226 days.

In this sample, 38 participants were classified as high grief and 73 participants were classified as low grief, on the basis of the established clinical cut score from the ICG. The participants in these subgroups had similar characteristics in terms of gender, BMI, income, education, physical activity, days since spouse's passing, and minutes since blood was drawn before the TSST; however, participants in the low-grief group were slightly older ($p = .003$) and had more comorbid conditions than those in the high-grief group ($p = .050$; see Table 2). Seventy-two percent of the variance in serum IL-6 could be explained by the person. The best-fitting model allowed the intercepts to vary by person ($p < .001$). When we assessed whether the slope between time and inflammation should randomly vary, we found that the best-fitting model allowed the slope between time and inflammation to randomly vary rather than be fixed, $\chi^2(2, N = 111) = 46.91$, $p < .001$. Thus, we report models with randomly varying slopes here. Last, we identified a positive linear relationship between time (modeled continuously in minutes) and serum IL-6, $t(109.7) = 8.66$, $p < .001$. Thus, a linear effect of time was included in each model here.

Table 1. Descriptive Statistics for and Correlations Between Key Study Variables

Variable	Descriptive statistic	Correlations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1. Average[a] log-transformed interleukin-6	*M* = 0.65 (0.92)													
2. Grief symptoms cut score (high grief)	*n* = 38 (34%) (0.47)	−.02 [−.13, .09]												
3. Grief symptoms (continuous)	*M* = 20.30 (12.13)	.02 [−.08, .13]	.84** [.81, .87]											
4. Depressive symptoms (continuous)	*M* = 13.67 (9.75)	−.07 [−.18, .04]	.64** [.57, .70]	.73** [.67, .77]										
5. Time (minutes after TSST)	*M* = 55.52 (50.47)	.25** [.14, .35]	.00 [−.11, .11]	−.00 [−.11, .11]	−.00 [−.11, .11]									
6. Age (years)	*M* = 68.04 (9.35)	.15** [.04, .25]	−.17** [−.27, −.06]	−.26** [−.36, −.15]	−.34** [−.43, −.24]	−.01 [−.12, .10]								
7. Gender (female)	*n* = 73 (65%) (0.48)	−.04 [−.15, .07]	−.05 [−.16, .06]	−.09 [−.19, .02]	.11 [−.00, .21]	−.02 [−.13, .09]	−.13* [−.23, −.02]							
8. Race[b]	*M* = 0.26 (0.90)	−.06 [−.17, .05]	.12* [.01, .23]	.21** [.10, .31]	.17** [.07, .28]	.01 [−.09, .12]	−.29** [−.39, −.19]	.13* [.02, .23]						
9. Education[c]	*M* = 0.72 (1.11)	.13* [.02, .23]	−.06 [−.16, .05]	−.04 [−.14, .07]	−.09 [−.20, .02]	−.00 [−.11, .11]	.06 [−.05, .17]	.02 [−.09, .13]	.01 [−.10, .12]					
10. Body mass index	*M* = 27.47 (5.18)	.28** [.18, .38]	−.05 [−.16, .06]	−.01 [−.11, .10]	−.02 [−.13, .09]	.00 [−.10, .11]	.02 [−.09, .13]	−.10 [−.20, .01]	.04 [−.07, .15]	.28** [.18, .38]				
11. Comorbid conditions	*M* = 0.35 (1.07)	.07 [−.04, .18]	−.11 [−.22, .00]	−.08 [−.18, .03]	−.06 [−.16, .05]	.00 [−.11, .11]	.08 [−.03, .19]	−.03 [−.14, .08]	−.06 [−.16, .05]	−.08 [−.19, .03]	−.06 [−.17, .05]			
12. Physical activity[d]	*M* = 2,737.82 (2,943.96)	−.05 [−.16, .06]	.02 [−.09, .13]	−.02 [−.13, .09]	−.03 [−.14, .08]	.01 [−.10, .11]	.10 [−.01, .21]	−.16** [−.26, −.05]	−.14* [−.24, −.03]	−.08 [−.18, .03]	−.05 [−.16, .06]	−.04 [−.15, .07]		
13. Days since spousal passing	*M* = 138.21 (16.90)	.10 [−.01, .21]	−.00 [−.11, .11]	−.03 [−.14, .08]	−.06 [−.17, .05]	−.01 [−.12, .10]	−.11* [−.22, −.00]	.03 [−.07, .14]	−.14* [−.24, −.03]	−.16** [−.27, −.05]	−.08 [−.18, .03]	.08 [−.03, .19]	−.04 [−.15, .07]	
14. Minutes before TSST	*M* = 24.75 (4.62)	−.22** [−.32, −.12]	.03 [−.08, .14]	−.08 [−.19, .03]	.01 [−.09, .12]	−.01 [−.12, .10]	.05 [−.06, .16]	−.02 [−.13, .09]	.00 [−.10, .11]	.10 [−.01, .20]	−.15** [−.25, −.04]	.13* [.02, .24]	−.03 [−.13, .08]	.04 [−.07, .15]

Note: For means in the Descriptive Statistic column, values in parentheses are standard deviations; for counts, the first value in parentheses is the percentage of the sample and the second is the standard deviation for that percentage. Values in brackets are 95% confidence intervals. The confidence interval is a plausible range of population correlations that could have caused the sample correlation (Cumming, 2014). TSST = Trier Social Stress Test.

[a]This value represents the average across each time point. [b]Participants reported their primary race by choosing from the following categories: 0 = White, 1 = Black or African American, 2 = Asian, 3 = Native Hawaiian or Pacific Islander, 4 = American Indian or Alaska Native, 5 = other. [c]Education was assessed using an ordinal scale ranging from 0 (indicating graduate or professional training) to 5 (indicating less than 7 years of schooling). [d]Total weekly physical activity was estimated by weighting the intensity of time spent in each activity with its estimated metabolic equivalent (MET) energy expenditure. Walking, moderate-intensity activity, and vigorous-intensity activity were assigned 3.3 METs, 4.0 METs, and 8.0 METs, respectively, and were used to calculate a total MET-minutes-per-week score to measure physical activity.

*$p < .05$. **$p < .01$.

Table 2. Descriptive Statistics for Demographic and Health Information Based on Grief-Symptom Severity

Characteristic	High-grief group (n = 38)	Low-grief group (n = 73)	p
Total physical activity[a]	M = 2,796.14 (2,495.13)	M = 2,670.20 (3,168.87)	.714
Age (years)	M = 66.29 (9.94)	M = 69.04 (9.04)	**.003**
Gender (female)	63% (0.49)	67% (0.47)	.355
Education[b]	M = 0.63 (1.13)	M = 0.78 (1.10)	.277
Body mass index	M = 27.06 (4.79)	M = 27.64 (5.42)	.349
Days since spousal passing	M = 137.47 (22.06)	M = 138.56 (14.10)	.957
Total family income[c]	M = 6.42 (1.90)	M = 6.71 (1.56)	.115
Minutes before TSST	M = 24.94 (3.45)	M = 24.71 (5.14)	.589
Charlson Comorbidity Index	M = 0.18 (0.46)	M = 0.42 (1.27)	**.050**

Note: Standard deviations are given in parentheses. p values for tests that revealed significant differences between conditions are given in boldface. TSST = Trier Social Stress Test.
[a]Total weekly physical activity was estimated by weighting the intensity of time spent in each activity with its estimated metabolic equivalent (MET) energy expenditure. Walking, moderate-intensity activity, and vigorous-intensity activity were assigned 3.3 METs, 4.0 METs, and 8.0 METs, respectively, and used to calculate a total MET-minutes-per-week score to measure physical activity. [b]Education was assessed using an ordinal scale ranging from 0 (indicating graduate or professional training) to 5 (indicating less than 7 years of schooling). [c]Total family income was assessed using an ordinal scale starting at 0 if the family did not have any income in the prior year. This scale ranges from 0 to 8 (1 = total family income between $5,000 and $11,999; 8 = income of $100,000 or greater).

Grief symptoms and IL-6 following the TSST

First, we evaluated the association between IL-6 and grief symptoms (i.e., ICG cut score) over time (across baseline, 45 min after the stressor, and 2 hr after the stressor) using a case-categorical approach (high or low grief) to determine whether these groups changed differently over the 2 hr after the stressor. There were no baseline differences in serum IL-6 before the TSST based on people's reported grief symptoms (categorical or continuous measures of grief symptoms; both ps > .62). We report each result unadjusted and adjusted for relevant covariates: time, age, gender, years of education, BMI, comorbidities, physical activity, days since spouse's passing, and minutes since blood was drawn before the TSST.

We found a significant interaction of grief-symptom group (high vs. low) and time (unadjusted: b = 0.14, p = .038; adjusted: b = 0.14, p = .035; see Table 3), controlling for fixed effects of the covariates. Specifically, participants in the high-grief group had a steeper rise in log IL-6 relative to those in the low-grief group. Examination of simple slopes indicated that participants in the high-grief group demonstrated a 46% increase in IL-6 per hour, exp(b) = 1.46, p < .001, whereas those in the low-grief group demonstrated a 26% increase, exp(b) = 1.26, p < .001. In other words, high grief was related to a 20% greater increase in IL-6 per hour than low grief. Modeling grief instead as a continuous variable did not yield the same level of support for the interaction (unadjusted: b = 0.00, p = .087; adjusted: b = 0.00, p = .083; see Table 4).

Because we were interested in whether grief symptoms predicted the rise in IL-6 above and beyond the influence of depression, we reran each of the above models with the same covariates and examined whether there was a difference in the results after including depressive symptoms (continuous) in the model. Results were unchanged: We found that the significant interaction of grief-symptom group (high vs. low) and time persisted (b = 0.14, p = .035; see Table 3), again controlling for fixed effects of the covariates. Specifically, participants in the high-grief group had a steeper rise in log IL-6 relative to those in the low-grief group. Simple-slopes follow-up tests confirmed that even after analyses adjusted for depressive symptoms, participants in the high-grief group demonstrated a 45% increase in IL-6 per hour, exp(b) = 1.45, p < .001, whereas those in the low-grief group demonstrated a 26% increase, exp(b) = 1.26, p < .001 (see Fig. 1). This similarly reflected that high grief was related to a 19% greater increase in IL-6 per hour than low grief. Again, modeling grief instead as a continuous variable did not yield the same level of support for the interaction (b = 0.00, p = .083). The strength of the estimate of the interaction (between grief-symptom group and time) and the level of significance was identical across adjusted models

Table 3. Results of the Regression Analysis of the Interaction of Grief Symptoms (Categorical) and Time in Unadjusted, Adjusted Without Depressive Symptoms, and Adjusted With Depressive Symptoms (Continuous) Models Using Imputation for Missing Data

	Interleukin-6 (unadjusted)[a]			Interleukin-6 (adjusted without depressive symptoms)[b]			Interleukin-6 (adjusted with depressive symptoms)[c]		
Predictor	*b*	95% CI	*p*	*b*	95% CI	*p*	*b*	95% CI	*p*
Intercept	0.66	[0.47, 0.85]	< **.001**	−1.32	[−3.37, 0.74]	.208	−1.25	[−3.39, 0.89]	.254
Grief (categorical) × Time	0.14	[0.01, 0.27]	**.038**	0.14	[0.01, 0.27]	**.035**	0.14	[0.01, 0.27]	**.035**
Grief (categorical)	−0.02	[−0.35, 0.31]	.899	0.07	[−0.23, 0.38]	.635	0.10	[−0.29, 0.49]	.610
Time	0.23	[0.15, 0.31]	< **.001**	0.23	[0.15, 0.30]	< **.001**	0.23	[0.15, 0.30]	< **.001**
Age				0.02	[0.00, 0.03]	**.029**	0.02	[0.00, 0.03]	**.046**
Gender				0.11	[−0.19, 0.41]	.456	0.12	[−0.18, 0.43]	.439
Education				0.02	[0.00, 0.03]	**.047**	0.01	[−0.00, 0.03]	.051
Body mass index				0.04	[0.01, 0.07]	**.004**	0.04	[0.01, 0.07]	**.004**
Comorbidities				0.07	[−0.07, 0.20]	.334	0.07	[−0.07, 0.20]	.332
Days since spouse's passing				0.01	[−0.00, 0.01]	.129	0.01	[−0.00, 0.01]	.139
Minutes before TSST				−0.05	[−0.08, −0.02]	**.001**	−0.05	[−0.08, −0.02]	**.001**
Physical activity				−0.00	[−0.00, 0.00]	.590	−0.00	[−0.00, 0.00]	.593
Depressive symptoms (continuous)							−0.00	[−0.02, 0.02]	.821

Note: *p* values for tests that revealed significant differences between conditions are given in boldface. CI = confidence interval; TSST = Trier Social Stress Test.

[a]For interleukin-6 (unadjusted), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.67$, $\tau_{11} = 0.07$, $\rho_{01} = -0.31$, intraclass correlation coefficient (ICC) = .90, $N = 111$ participants, $N = 325$ observations, marginal $R^2 = .067$, conditional $R^2 = .904$. [b]For interleukin-6 (adjusted without depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.54$, $\tau_{11} = 0.07$, $\rho_{01} = -0.25$, ICC = .88, $N = 111$ participants, $N = 325$ observations, marginal $R^2 = .238$, conditional $R^2 = .907$. [c]For interleukin-6 (adjusted with depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.55$, $\tau_{11} = 0.07$, $\rho_{01} = -0.25$, ICC = .88, $N = 111$ participants, $N = 325$ observations, marginal $R^2 = .236$, conditional $R^2 = .908$.

with and without (continuous) depressive symptoms (see Tables 3 and 4; see Tables S1 and S2 in the Supplemental Material for results including the cut score for clinically significant depressive symptoms).

Sensitivity analysis

In an ancillary analysis, we examined whether there was a difference in the models when we did not impute missing data in our predictor variables. Overall, the findings remained consistent when we employed listwise deletion instead of random-forest imputation to account for missing data. Thus, the following analyses are based on the 101 participants who had no missing predictor data.

We found that the significant interaction of grief-symptom group (high vs. low) and time persisted ($b = 0.17$, ps = .017–.018; see Table 5), again controlling for fixed effects of the covariates, including depressive symptoms. Specifically, participants in the high-grief group had a steeper rise in log IL-6 relative to those in the low-grief group. Simple-slopes follow-up tests confirmed that even after analyses adjusted for depressive symptoms, participants in the high-grief group demonstrated a 49% increase in IL-6 per hour, $\exp(b) = 1.49$, $p < .001$, whereas those in the low-grief group demonstrated a 25% increase, $\exp(b) = 1.25$, $p < .001$. Here, high grief was related to a 24% greater increase in IL-6 per hour than low grief.

In these listwise-deletion models, we found that the interaction between grief (modeled as a continuous rather than categorical variable) and time was statistically significant ($b = 0.01$, ps = .042–.044; see Table 6) after controlling for each covariate, including depressive symptoms. Specifically, participants who reported higher grief symptoms had a steeper rise in log IL-6 relative to those who reported lower grief symptoms. Follow-up tests of simple slopes within different quartile ranges ($< Q_1$, $Q_1 - Q_3$, $> Q_3$) followed the same pattern of results found using the categorical measure of grief symptoms. Specifically, participants who reported more grief symptoms experienced a steeper increase in IL-6 per hour: $< Q_1$: $\exp(b) = 1.18$, $p = .022$; $Q_1 - Q_3$: $\exp(b) = 1.35$, $p < .001$; $> Q_3$: $\exp(b) = 1.43$, $p < .001$, respectively.

Discussion

When exposed to an acute experimental stressor, bereaved spouses who reported high grief symptoms produced an inflammatory response (indexed by

Table 4. Results of the Regression Analysis of the Interaction of Grief Symptoms (Continuous) and Time in Unadjusted, Adjusted Without Depressive Symptoms, and Adjusted With Depressive Symptoms (Continuous) Models Using Imputation for Missing Data

	Interleukin-6 (unadjusted)[a]			Interleukin-6 (adjusted without depressive symptoms)[b]			Interleukin-6 (adjusted with depressive symptoms)[c]		
Predictor	*b*	95% CI	*p*	*b*	95% CI	*p*	*b*	95% CI	*p*
Intercept	0.61	[0.30, 0.91]	< **.001**	−1.55	[−3.68, 0.59]	.156	−1.47	[−3.63, 0.68]	.180
Grief (continuous) × Time	0.00	[−0.00, 0.01]	.087	0.00	[−0.00, 0.01]	.083	0.00	[−0.00, 0.01]	.083
Grief (continuous)	0.00	[−0.01, 0.02]	.734	0.00	[−0.01, 0.02]	.450	0.01	[−0.01, 0.03]	.348
Time	0.19	[0.07, 0.31]	**.003**	0.18	[0.06, 0.31]	**.003**	0.18	[0.06, 0.31]	**.003**
Age				0.02	[0.00, 0.03]	**.023**	0.02	[0.00, 0.03]	**.038**
Gender				0.12	[−0.18, 0.43]	.418	0.15	[−0.16, 0.45]	.356
Education				0.02	[0.00, 0.03]	**.046**	0.01	[−0.00, 0.03]	.054
Body mass index				0.04	[0.01, 0.07]	**.004**	0.04	[0.01, 0.07]	**.004**
Comorbidities				0.06	[−0.07, 0.19]	.351	0.06	[−0.07, 0.20]	.350
Days since spouse's passing				0.01	[−0.00, 0.01]	.113	0.01	[−0.00, 0.01]	.126
Minutes before TSST				−0.05	[−0.08, −0.02]	**.001**	−0.05	[−0.08, −0.02]	**.002**
Physical activity				−0.00	[−0.00, 0.00]	.613	−0.00	[−0.00, 0.00]	.630
Depressive symptoms (continuous)							−0.01	[−0.03, 0.02]	.564

Note: *p* values for tests that revealed significant differences between conditions are given in boldface. CI = confidence interval; TSST = Trier Social Stress Test.

[a]For interleukin-6 (unadjusted), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.67$, $\tau_{11} = 0.07$, $\rho_{01} = -0.32$, intraclass correlation coefficient (ICC) = .90, N = 111 participants, N = 325 observations, marginal R^2 = .067, conditional R^2 = .904. [b]For interleukin-6 (adjusted without depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.54$, $\tau_{11} = 0.07$, $\rho_{01} = -0.25$, ICC = .88, N = 111 participants, N = 325 observations, marginal R^2 = .238, conditional R^2 = .907. [c]For interleukin-6 (adjusted with depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.54$, $\tau_{11} = 0.07$, $\rho_{01} = -0.25$, ICC = .88, N = 111 participants, N = 325 observations, marginal R^2 = .238, conditional R^2 = .907.

the cytokine IL-6) that was 19% larger than the response in those who reported low grief symptoms. Grief was associated with the rise in serum IL-6 over and above the influence of depressive symptoms.

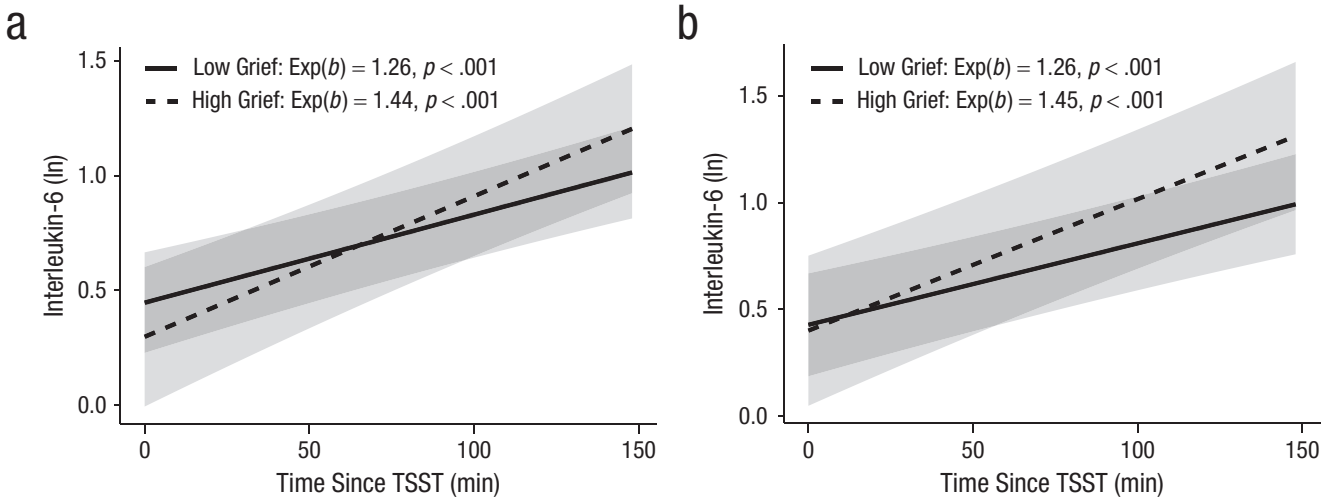

Fig. 1. Estimates of the effect of high and low grief symptoms on inflammation following the Trier Social Stress Test (TSST), separately for models (a) using only time as a predictor and (b) including all covariates. Shaded regions represent 95% confidence intervals. Estimates were based on standard errors generated by the *predict()* function in R (R Core Team, 2019). Simple slopes for each group are also presented. Because the outcome was log-transformed, regression coefficients were exponentiated to provide an index of the percentage of change in the raw units of the outcome.

Table 5. Results of the Regression Analysis of the Interaction of Grief Symptoms (Categorical) and Time in Unadjusted, Adjusted Without Depressive Symptoms, and Adjusted With Depressive Symptoms (Continuous) Models Using Listwise Deletion for Missing Data ($N = 101$)

	Interleukin-6 (unadjusted)[a]			Interleukin-6 (adjusted without depressive symptoms)[b]			Interleukin-6 (adjusted with depressive symptoms)[c]		
Predictor	*b*	95% CI	*p*	*b*	95% CI	*p*	*b*	95% CI	*p*
Intercept	0.66	[0.47, 0.85]	< **.001**	−2.67	[−4.64, −0.71]	**.008**	−2.69	[−4.75, −0.63]	**.011**
Grief (categorical) × Time	0.14	[0.01, 0.27]	**.038**	0.17	[0.03, 0.30]	**.017**	0.17	[0.03, 0.30]	**.018**
Grief (categorical)	−0.02	[−0.35, 0.31]	.899	0.03	[−0.29, 0.35]	.839	0.03	[−0.40, 0.45]	.903
Time	0.23	[0.15, 0.31]	< **.001**	0.23	[0.15, 0.31]	< **.001**	0.23	[0.15, 0.31]	< **.001**
Age				0.02	[0.00, 0.03]	**.030**	0.02	[0.00, 0.04]	**.036**
Gender				0.12	[−0.20, 0.44]	.453	0.12	[−0.20, 0.45]	.454
Education				0.05	[−0.09, 0.20]	.458	0.05	[−0.09, 0.20]	.469
Body mass index				0.04	[0.01, 0.07]	**.017**	0.04	[0.01, 0.07]	**.019**
Comorbidities				0.12	[−0.07, 0.31]	.223	0.12	[−0.08, 0.31]	.231
Days since spouse's passing				0.01	[−0.00, 0.02]	.106	0.01	[−0.00, 0.02]	.113
Minutes before TSST				−0.05	[−0.09, −0.02]	**.001**	−0.05	[−0.09, −0.02]	**.001**
Physical activity				−0.00	[−0.00, 0.00]	.713	−0.00	[−0.00, 0.00]	.708
Depressive symptoms (continuous)							0.00	[−0.02, 0.02]	.942

Note: *p* values for tests that revealed significant differences between conditions are given in boldface. CI = confidence interval; TSST = Trier Social Stress Test.

[a]For interleukin-6 (unadjusted), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.67$, $\tau_{11} = 0.07$, $\rho_{01} = -0.31$, intraclass correlation coefficient (ICC) = .90, $N = 111$ participants, $N = 325$ observations, marginal $R^2 = .067$, conditional $R^2 = .904$. [b]For interleukin-6 (adjusted without depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.55$, $\tau_{11} = 0.07$, $\rho_{01} = -0.22$, ICC = .88, $N = 102$ participants, $N = 298$ observations, marginal $R^2 = .248$, conditional $R^2 = .908$. [c]For interleukin-6 (adjusted with depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.56$, $\tau_{11} = 0.07$, $\rho_{01} = -0.22$, ICC = .88, $N = 101$ participants, $N = 297$ observations, marginal $R^2 = .245$, conditional $R^2 = .909$.

The current study findings, that grief symptoms were associated with IL-6 over and above depressive symptoms, add evidence to recent neuroscience developments suggesting that a unique neurobiological profile may exist for individuals experiencing extreme grief (Kakarala et al., 2020). In particular, the breaking of an attachment bond (i.e., the death of one's spouse; Stroebe et al., 2005) may partially explain the importance of grief symptoms over and above depressive symptoms after the death of a spouse. In adulthood, spouses play a critical role in maintaining physiological homeostasis (i.e., "coregulation"; Sbarra & Hazan, 2008, p. 143). Thus, a spouse's death disrupts homeostatic maintenance, leading to a dysregulation of multiple biological and physiological systems (for a review, see LeRoy et al., 2019). Recently bereaved spouses who experience more grief symptoms may exhibit subsequently higher proinflammatory cytokines (e.g., IL-6).

Bereaved spouses are also at heightened risk of cardiovascular disease (Shor et al., 2012). Thus, it is significant that grief symptoms were associated with a rise in inflammation, because inflammation is an important mediator and causal factor in developing coronary heart disease (Sarwar et al., 2012; Swerdlow et al., 2012). Broadly, an overactive inflammatory network promotes many diseases of older adulthood (e.g., Type 2 diabetes, Alzheimer's disease, osteoporosis, rheumatoid arthritis, periodontal disease, some cancers, and cardiovascular disease; Ershler & Keller, 2000; Rea et al., 2018). Because IL-6 is a central immunological mechanism involved in the onset and progression of many age-related diseases (Hunter & Jones, 2015), these findings may help explain the "widowhood effect," which refers to the increased risk for premature mortality among widowers (Moon et al., 2014).

The bereaved spouses in our sample, on average, reported considerable levels of grief and depressive symptoms. Specifically, our participants reported higher levels of depressive symptoms compared with past estimates of depression among nonbereaved community-dwelling older adults (Lewinsohn et al., 1997). Whereas the average depression score among nonbereaved community-dwelling older adults is approximately 8 points below the established cut score of 16 on the CES-D (Lewinsohn et al., 1997; Radloff, 1977), the average depression score for bereaved spouses in the current sample was only 2 points below the cut score of

Table 6. Results of the Regression Analysis of the Interaction of Grief Symptoms (Continuous) and Time in Unadjusted, Adjusted Without Depressive Symptoms, and Adjusted With Depressive Symptoms (Continuous) Models Using Listwise Deletion for Missing Data ($N = 101$)

	IL-6 (unadjusted)[a]			IL-6 (adjusted without depressive symptoms)[b]			IL-6 (adjusted with depressive symptoms)[c]		
Predictor	*b*	95% CI	*p*	*b*	95% CI	*p*	*b*	95% CI	*p*
Intercept	0.61	[0.30, 0.91]	< **.001**	−2.86	[−4.90, −0.82]	**.006**	−2.82	[−4.89, −0.75]	**.008**
Grief (continuous) × Time	0.00	[−0.00, 0.01]	.087	0.01	[0.00, 0.01]	**.042**	0.01	[0.00, 0.01]	**.044**
Grief (continuous)	0.00	[−0.01, 0.02]	.734	0.00	[−0.01, 0.02]	.556	0.01	[−0.01, 0.03]	.483
Time	0.19	[0.07, 0.31]	**.003**	0.17	[0.04, 0.30]	**.010**	0.17	[0.04, 0.30]	**.010**
Age				0.02	[0.00, 0.04]	**.024**	0.02	[0.00, 0.04]	**.030**
Gender				0.14	[−0.18, 0.45]	.405	0.16	[−0.17, 0.49]	.348
Education				0.05	[−0.09, 0.20]	.449	0.05	[−0.09, 0.19]	.486
BMI				0.04	[0.01, 0.07]	**.017**	0.04	[0.01, 0.07]	**.018**
Comorbidities				0.12	[−0.07, 0.31]	.225	0.12	[−0.08, 0.31]	.240
Days since spouse's passing				0.01	[−0.00, 0.02]	.093	0.01	[−0.00, 0.02]	.109
Minutes before TSST				−0.05	[−0.08, −0.02]	**.001**	−0.05	[−0.08, −0.02]	**.002**
Physical activity				−0.00	[−0.00, 0.00]	.729	−0.00	[−0.00, 0.00]	.722
Depressive symptoms (continuous)							−0.00	[−0.03, 0.02]	.692

Note: *p* values for tests that revealed significant differences between conditions are given in boldface. CI = confidence interval; TSST = Trier Social Stress Test.

[a]For interleukin-6 (unadjusted), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.67$, $\tau_{11} = 0.07$, $\rho_{01} = -0.32$, intraclass correlation coefficient (ICC) = .90, $N = 111$ participants, $N = 325$ observations, marginal $R^2 = .067$, conditional $R^2 = .904$. [b]For interleukin-6 (adjusted without depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.55$, $\tau_{11} = 0.07$, $\rho_{01} = -0.23$, ICC = .88, $N = 102$ participants, $N = 298$ observations, marginal $R^2 = .248$, conditional $R^2 = .908$. [c]For interleukin-6 (adjusted with depressive symptoms), random effects are $\sigma^2 = 0.08$, $\tau_{00} = 0.56$, $\tau_{11} = 0.07$, $\rho_{01} = -0.24$, ICC = .88, $N = 101$ participants, $N = 297$ observations, marginal $R^2 = .245$, conditional $R^2 = .909$.

16 (Radloff, 1977). Participants' average grief score was just below the cut score for complicated grief; however, given that these participants' spouses had passed away only 4 months previously, the present scores should not be interpreted as diagnostic for complicated grief (Prigerson et al., 1995).

We do not know what participants' levels of depressive symptoms were before the death of their spouse, which is a limitation of the study. It will be important to determine whether people's inflammatory responses to acute stress during bereavement differ on the basis of their history of depression or their prebereavement levels of depressive symptoms. Similarly, an important next step will involve designing studies specifically to disentangle the relationships between grief, depression, and IL-6, because the overlapping variance between grief and depression complicates our understanding of grief as an independent driver of these effects. We did not collect data on neuroendocrine markers (e.g., cortisol), but this would be a valuable addition to studies in the future. Future studies would also benefit from probing the spouse's cause of death (i.e., whether it was expected or unexpected) to determine whether the anticipation (or lack of warning) may affect the relationship between grief symptoms and stress-induced inflammation. Our study was also limited by the relatively high socioeconomic status and predominantly female composition of our sample. However, women are more likely to be widows than men are to be widowers (U.S. Census Bureau, 2019). Another limitation of the study is that the sample was predominantly White and non-Hispanic. Future research should aim to assess the generalizability of these findings and examine these relationships in samples more representative of the United States as a whole. Additionally, future research should focus on loss dynamics among Black Americans specifically, because Black Americans are more than twice as likely to lose a spouse by age 60 compared with White Americans (Umberson et al., 2017), and there is a paucity of bereavement research focusing on the loss of close relationships within Black families and communities (Umberson, 2017).

These limitations are balanced by several strengths, primarily having a well-defined sample at 4 months of bereavement and the use of a very well-established acute lab stressor, which will be important to inform future discussions around complicated grief. Finally, future work can expand on this study by administering

the TSST multiple times during spousal bereavement to assess whether changes in grief symptoms are also accompanied by changes in stress reactivity; this will be an important step in determining causality.

Recently bereaved spouses with high grief symptoms exhibited enhanced inflammation to an acute laboratory stressor compared with recently bereaved spouses with fewer grief symptoms. Accordingly, grief symptoms may enhance the stress-response system to promote excessive inflammation. These findings add to our emerging understanding of bereavement, grief, and immune dysregulation.

Transparency

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Author Contributions

C. P. Fagundes and C. Heijnen developed the parent study concept and study design; C. P. Fagundes oversaw all aspects of data collection and manuscript preparation. J. Liu performed immune assays for the parent study, which includes the data presented here. R. L. Brown began the investigation for this specific article and analyzed and interpreted the data under the supervision of C. P. Fagundes and R. Suchting. R. L. Brown and A. S. LeRoy drafted the manuscript, and M. A. Chen, L. M. Jaremka, and C. P. Fagundes provided critical revisions. All authors approved the final manuscript for submission.

Declaration of Conflicting Interests

The author(s) declared that there were no conflicts of interest with respect to the authorship or the publication of this article.

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Open Practices

Data and materials for this study have not been made publicly available, and the design and analysis plans were not preregistered.

ORCID iDs

Ryan L. Brown https://orcid.org/0000-0003-2844-5928
Christopher P. Fagundes https://orcid.org/0000-0003-3173-2215

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Supplemental Material

Additional supporting information can be found at http://journals.sagepub.com/doi/suppl/10.1177/09567976211059502

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